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(71) Applicant: PROGENITOR, INC. [US/US]; 1507 Chambers Road, Columbus, OH 43212 (US).

(72) Inventors: SNODGRASS, H., Ralph; 650 Retreat Lane, Powell, OH 43065 (US). CIOFFI, Joseph; 1180 Bayboro Drive, New Albany, OH 43054 (US). ZUPANCIC, Thomas, J.; 501 Park Boulevard, Worthington, OH 43085 (US). SHAFER, Alan, W.; 256 Lakeview Drive, Lancaster, OH 43130 (US).

(74) Agents: POISSANT, Brian, M. et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).

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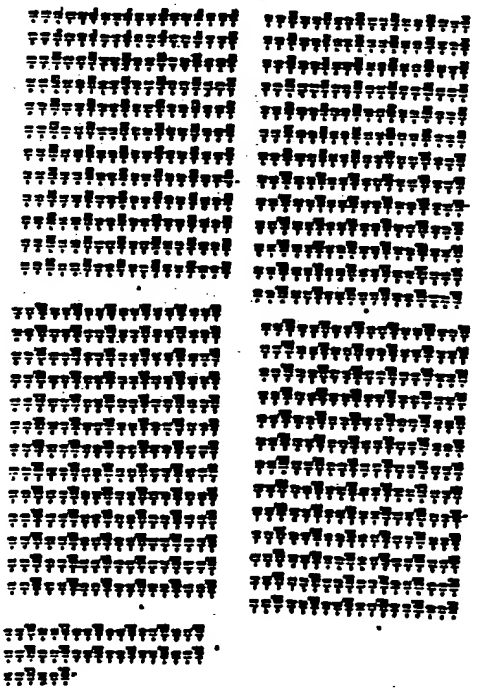
(57) Abstract

The present invention relates to a variant form of the receptor for the *obese* gene product. In particular, the invention relates to methods of detecting this receptor variant in cells and tissues of obese individuals. In addition, it relates to methods of inhibiting or down-regulating expression of this variant in cells to augment their responsiveness to weight regulation by leptin as well as methods of using compounds to directly activate signal transduction pathways associated with this ligand-receptor system.



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DETECTION OF A LEPTIN RECEPTOR VARIANT AND METHODS FOR REGULATING OBESITY

1. INTRODUCTION

The present invention relates to a variant form of the receptor for the *obese* gene product. In particular, the invention relates to methods of detecting this receptor variant in cells and tissues of obese individuals. In addition, it relates to methods of inhibiting or down-regulating expression of this variant in cells to augment their responsiveness to weight regulation by leptin as well as methods of using compounds to directly activate signal transduction pathways associated with this ligand-receptor system.

2. BACKGROUND OF THE INVENTION

Obesity is not only a nutritional disorder in Western societies, it is also a serious health concern because of its association with adult-onset diabetes, hypertension, and heart disease (Grundy, 1990, *Disease-a-Month* 36:645-696). While there was evidence to suggest that body weight was physiologically regulated, the molecular mechanism has remained elusive. However, animal studies have produced several mouse strains that contain single-gene mutations, resulting in an obese phenotype. One such recessive mutation is manifested in the *ob/ob* mice, and it is referred to as the *obese* (*ob*) mutation.

Zhang et al. (1994, *Nature* 372:425-432) describe the cloning and sequencing of the mouse *ob* gene and its human homolog. When an isolated gene fragment was used as a probe, it was shown to hybridize with RNA only in white adipose tissue by northern blot analysis, but no expression was detected in any other tissue. In addition, the coding sequence of the *ob* gene hybridized to all vertebrate genomic DNAs tested, indicating a high level of conservation of this molecule among vertebrates. The deduced amino acid sequences

are 84% identical between human and mouse, and both molecules contain features of secreted proteins.

In an effort to understand the physiologic function of the *ob* gene, several independent research groups produced recombinant *ob* gene product in bacteria for *in vivo* testing (Pelleymounter et al., 1995, *Science* 269:540-543; Halaas et al., 1995, *Science* 269:543-546; Campfield et al., 1995, *Science* 269:546-549). When the Ob protein (also known as leptin) was injected into grossly obese mice, which possessed two mutant copies of the *ob* gene, the mice exhibited a reduced appetite and began to lose weight. In addition, these studies described a dual action of leptin in both reducing the animals' food intake and in increasing their energy expenditure. Similarly, when normal mice received leptin, they also ate less than the untreated controls. More importantly, Campfield et al. (1995, *Science* 269:546-549) injected leptin directly into lateral ventricle, and observed a reduction in the animals' food intake, suggesting that leptin acts on central neuronal networks to regulate feeding behavior and energy balance. Thus, this result provides evidence that the leptin receptor (also known as OB-R) is expressed by cells in the brain.

Recently, a leptin fusion protein was generated and used to screen for OB-R in a cDNA expression library prepared from mouse choroid plexus, a tissue that lines brain cavities termed ventricles (Tartalia, 1995, *Cell* 83:1263-1271). This approach led to the cloning of one form of the OB-R coding sequence, which reveals a single membrane-spanning receptor, sharing structural similarities with several Class I cytokine receptors, such as the gp130 signal-transducing component of the interleukin-6 receptor (Taga et al., 1989, *Cell* 58:573-581), the granulocyte-colony stimulating factor receptor (Fukunaga et al., 1990, *Cell* 61:341-350), and the leukemia inhibitory factor receptor (Gearing et al., 1991, *EMBO J.* 10:2839-2848). Northern blot analysis and reverse transcription-polymerase chain reaction (RT-PCR) demonstrate

that OB-R mRNA is expressed in several tissues, including lung, kidney, total brain, choroid plexus and hypothalamus.

The reported mouse OB-R protein contains a relatively short intracellular cytoplasmic domain as compared with other Class I cytokine receptors. Subsequently, when cDNA encoding its human homolog was isolated from a human infant brain library, the predicted human protein sequence contains a much longer intracellular domain. In view of this finding, it was speculated that different forms of the receptor might exist (Barinaga, 1996, *Science* 271:29). However, prior to the present invention, there was no report on the identification of any variant forms of the OB-R in humans or how such molecules, if they exist, would relate to obesity.

Additionally, several studies have shown that *ob* gene expression is actually increased in obese humans (Considine et al., 1995, *J. Clin. Invest.* 95:2986-2988; Lonnquist et al., 1995, *Nature Med.* 1:950; Hamilton et al., 1995, *Nature Med.* 1:953). Moreover, the mutations in the mouse *Ob* gene were not detected in human mRNA. Therefore, taken collectively, these studies imply that decreased leptin levels are not the primary cause of obesity, and argue for the presence of a less responsive receptor in obese individuals. There remains a need to isolate such an OB-R variant for the design of therapeutics to augment weight regulation by leptin.

3. SUMMARY OF THE INVENTION

The present invention relates to a variant form of the human OB-R. In particular, it relates to the detection of this receptor variant in cells of obese individuals, and methods for treating obesity by targeting this variant.

The invention is based, in part, upon the Applicants' discovery of human cDNA clones encoding a variant form of the OB-R. This receptor differs structurally from a reported OB-R with only three amino acid substitutions in the extracellular domain, but extensive diversity is observed in their intracellular cytoplasmic domains at the 3' end. The

cytoplasmic domain of the variant of the invention is both shorter and distinct in nucleotide sequence from the corresponding domain of the published form of OB-R. Therefore, a wide variety of uses are encompassed by the present invention, including but not limited to, the detection of the receptor variant in cells of obese individuals, methods to inhibit and/or down-regulate the expression of this receptor variant, gene therapy to replace the receptor variant in homozygous individuals, and direct activation of downstream signal transduction pathways in cells expressing the receptor variant for weight regulation.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-1E. Nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO: 2, 3 and 4) of the human OB-R variant. The amino acid sequence diverges from the human OB-R reported by Tartaglia et al. (1995, *Cell* 83:1263-1271) at nucleotide residue #349, #422, #764 and from residue #2770 and beyond.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1. THE OB-R VARIANT

The present invention relates to nucleic acid and amino acid sequences of an OB-R variant in the Class I cytokine receptor family. In a specific embodiment by way of example in Section 6, *infra*, this variant was cloned and characterized. Amino acid sequence comparison of this OB-R variant with a published human OB-R sequence (Tartaglia et al., 1995, *Cell* 83:1263-1271) reveals three amino acid differences in their extracellular domain and extensive diversity in their intracellular cytoplasmic domains. More specifically, Figure 1A-1E shows that in the variant, nucleotide residues #349-351 encode alanine, nucleotide residues #421-423 encode arginine and nucleotide residues #763-765 encode arginine. Additionally, the variant diverges both in length and sequence composition from the published human OB-R sequence from nucleotide residue #2770 and beyond.

In order to clone additional variant forms of the molecule, labeled DNA probes made from nucleic acid fragments corresponding to any portion of the cDNA disclosed herein may be used to screen a cDNA library prepared from human fetal liver, human lung, human kidney, human choroid plexus and human hypothalamus. More specifically, oligonucleotides corresponding to either the 5' or 3' terminus of the cDNA sequence may be used to obtain longer nucleotide sequences. Briefly, the library may be plated out to yield a maximum of 30,000 pfu for each 150 mm plate. Approximately 40 plates may be screened. The plates are incubated at 37°C until the plaques reach a diameter of 0.25 mm or are just beginning to make contact with one another (3-8 hours). Nylon filters are placed onto the soft top agarose and after 60 seconds, the filters are peeled off and floated on a DNA denaturing solution consisting of 0.4N sodium hydroxide. The filters are then immersed in neutralizing solution consisting of 1M Tris HCL, pH 7.5, before being allowed to air dry. The filters are prehybridized in casein hybridization buffer containing 10% dextran sulfate, 0.5M NaCl, 50mM Tris HCL, pH 7.5, 0.1% sodium pyrosphosphate, 1% casein, 1% SDS, and denatured salmon sperm DNA at 0.5 mg/ml for 6 hours at 60°C. The radiolabelled probe is then denatured by heating to 95°C for 2 minutes and then added to the prehybridization solution containing the filters. The filters are hybridized at 60°C for 16 hours. The filters are then washed in 1X wash mix (10X wash mix contains 3M NaCl, 0.6M Tris base, and 0.02M EDTA) twice for 5 minutes each at room temperature, then in 1X wash mix containing 1% SDS at 60°C for 30 minutes, and finally in 0.3X wash mix containing 0.1% SDS at 60°C for 30 minutes. The filters are then air dried and exposed to x-ray film for autoradiography. After developing, the film is aligned with the filters to select a positive plaque. If a single, isolated positive plaque cannot be obtained, the agar plug containing the plaques will be removed and placed in lambda dilution buffer containing 0.1M NaCl, 0.01M magnesium sulfate, 0.035M Tris HCl, pH 7.5, 0.01% gelatin. The phage

may then be replated and rescreened to obtain single, well isolated positive plaques. Positive plaques may be isolated and the cDNA clones sequenced using primers based on the known cDNA sequence. This step may be repeated until a full length cDNA is obtained.

One method for identifying all 3' isoforms is to PCR amplify the 3' ends of the variant cDNA from a variety of tissues including but not limiting to, choroid plexus, hypothalamus, fetal liver, bone marrow, ovary, or prostate. To obtain the 3' end of the cDNA, an oligo-dT primer is used to synthesize the cDNA first strand. OB-R specific primers from the conserved region of the gene (e.g. up stream of nucleotide 2770) and oligo-dT are then used to amplify the 3' end. The PCR fragments are cloned and sequenced by standard techniques. Once obtained, these sequences may be translated into amino acid sequence and examined for certain landmarks such as continuous open reading frame, regulatory regions that associate with tyrosine kinase activation, and finally overall structural similarity to known OB-R variants.

5.2. EXPRESSION OF THE OB-R VARIANT

In accordance with the invention, the OB-R variant polynucleotide sequence which encodes a protein, peptide fragments, fusion proteins or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of the protein, peptide fragments, fusion proteins or a functional equivalent thereof, in appropriate host cells. Such polynucleotide sequences, as well as other polynucleotides which selectively hybridize to at least a part of such polynucleotides or their complements, may also be used in nucleic acid hybridization assays, Southern and Northern blot analyses, etc.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used in the practice of the invention for the expression of the OB-R variant. Such DNA sequences include those which are capable

of hybridizing to the OB-R variant sequence under stringent conditions, particularly at its 3' end. The phrase "stringent conditions" as used herein refers to those hybridizing conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Altered DNA sequences which may be used in accordance with the invention include deletions, additions or substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. The gene product itself may contain deletions, additions or substitutions of amino acid residues within the OB-R variant sequence, which result in a silent change thus producing a functionally equivalent protein. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine, histidine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: glycine, asparagine, glutamine, serine, threonine, tyrosine; and amino acids with nonpolar head groups include alanine, valine, isoleucine, leucine, phenylalanine, proline, methionine, tryptophan.

The DNA sequence of the invention may be engineered in order to alter the OB-R variant coding sequence for a variety of ends, including but not limited to, alterations which

modify processing and expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, *e.g.*, site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, phosphorylation, etc. In addition, the intracellular domain may also be altered and replaced by a different domain, such as the OB-R intracellular domain by Tartaglia et al.

In another embodiment of the invention, the OB-R variant sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors or stimulators of receptor activity, it may be useful to encode a chimeric protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the OB-R variant sequence and the heterologous protein sequence, so that the variant may be cleaved away from the heterologous moiety.

In an alternate embodiment of the invention, the coding sequence of the OB-R variant could be synthesized in whole or in part, using chemical methods well known in the art. (See, for example, Caruthers *et al.*, 1980, *Nuc. Acids Res. Symp. Ser.* 7:215-233; Crea and Horn, 180, *Nuc. Acids Res.* *9(10)*:2331; Matteucci and Caruthers, 1980, *Tetrahedron Letters 21*:719; and Chow and Kempe, 1981, *Nuc. Acids Res.* *9(12)*:2807-2817). Alternatively, the protein itself could be produced using chemical methods to synthesize OB-R variant amino acid sequence in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (*e.g.*, see Creighton, 1983, *Proteins Structures And Molecular Principles*, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 34-49).

In order to express the OB-R variant in host cells, the nucleotide sequence coding for the variant, or a functional equivalent, is inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. The expressed gene products as well as host cells or cell lines transfected or transformed with recombinant OB-R variant expression vectors can be used for a variety of purposes. For example, host cells expressing the OB-R variant may be used to verify the ability of this molecule to bind leptin in a binding assay with radiolabeled, enzyme-conjugated or fluorescent dye-conjugated leptin. At the same time, the ability of the molecule to transduce an activation signal in host cells upon binding to leptin may be tested by assaying proliferation or phosphorylation pattern of kinases in the cells. In addition, genetically-engineered host cells can be used to screen for and select agonist and antagonist compounds, including any inhibitors that would interfere with binding of leptin to the extracellular domain of the OB-R variant. In that connection, such host cells may be used to screen for and select small molecules that can supplement the incomplete signal transduced by the OB-R variant following leptin binding. Other uses, include, but are not limited to generating antibodies (*i.e.*, monoclonal or polyclonal) that competitively inhibit activity of an OB-R variant, neutralize its activity, or even enhances it activity. Antibodies may be used in detecting and quantifying expression of OB-R levels in cells and tissues.

5.3. USES OF THE OB-R VARIANT POLYNUCLEOTIDE

The OB-R variant polynucleotide may be used for diagnostic and/or therapeutic purposes. For diagnostic purposes, the OB-R variant polynucleotide may be used to detect gene expression or aberrant gene expression in obese individuals as well as in normal individuals to identify predisposition for obesity. Included in the scope of the invention are oligonucleotide sequences, that include

antisense RNA and DNA molecules, ribozymes and triplex DNA, that function to inhibit translation of OB-R variant.

5.3.1. DIAGNOSTIC USES OF OB-R VARIANT POLYNUCLEOTIDE

The OB-R variant polynucleotide may have a number of uses for the diagnosis of the possible causes underlying obesity, resulting from expression of the receptor variant. For example, the OB-R variant cytoplasmic domain DNA sequence may be used in hybridization assays of biopsies or autopsies to diagnose OB-R variant expression; *e.g.*, Southern or Northern analysis, including *in situ* hybridization assays as well as PCR. Such techniques are well known in the art, and are in fact the basis of many commercially available diagnostic kits. For PCR detection, primers may be designed from a conserved region of the coding sequence and within the 3' region of OB-R variant. The tissues suitable for such analysis include but are not limited to, hypothalamus, choroid plexus, adipose tissues, lung, prostate, ovary, small intestine, bone marrow and peripheral blood mononuclear cells.

5.3.2. THERAPEUTIC USES OF THE OB-R VARIANT POLYNUCLEOTIDE

The OB-R variant polynucleotide may be useful in the treatment of various abnormal obese conditions. By introducing gene sequences into cells, gene therapy can be used to treat conditions in which the cells do not respond to leptin normally due to expression of the OB-R variant. In some instances, the polynucleotide encoding a functional OB-R is intended to replace or act in the place of the functionally deficient OB-R variant gene. Alternatively, abnormal conditions characterized by expression of two copies of the OB-R variant can be treated using the gene therapy techniques described below.

Non-responsiveness to normal levels of leptin is an important cause of obesity. This may result from a functionally defective receptor that does not transduce

competent signals upon ligand binding. Recombinant gene therapy vectors, such as viral vectors, may be engineered to express signalling competent forms of OB-R which may be used to augment the non-responsiveness of the naturally occurring OB-R variant. A signalling competent form may be, for example, a protein with the same extracellular domain and transmembrane region, but containing all or part of its normal signal transduction domain, such as that described by Tartaglia et al. (1995, *Cell* 83:1263-1271). Thus recombinant gene therapy vectors may be used therapeutically for treatment of obesity resulting from expression or activity of the OB-R variant. Accordingly, the invention provides a method of augmenting signal transduction by an endogenous OB-R variant in a cell comprising delivering a DNA molecule encoding a signalling competent form of the OB-R to the cell so that the signalling competent protein is produced in the cell and competes with the endogenous defective OB-R variant for access to molecules in the signalling pathway which does not activate or are not activated by the endogenous natural defective receptor. Additionally, since dimerization of a functional receptor with a defective variant may occur in cells of heterozygous individuals, small molecules may be used to inhibit such pairing, thereby increasing the number of functional dimeric receptors for proper signalling in response to leptin.

In contrast, overexpression of either leptin or a competent OB-R may result in a clinical anorexic-like syndrome due to a loss of appetite or hypermetabolic activity. In such cases, the OB-R variant of the invention may be introduced into cells with functional receptors to cause a decrease in the number of functional receptors or to compete with such receptors for leptin binding.

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of recombinant functional OB-R into the targeted cell population. Methods which are well known to those skilled in

the art can be used to construct recombinant viral vectors containing an OB-R polynucleotide sequence. See, for example, the techniques described in Sambrook *et al.*, 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel *et al.*, 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y. Alternatively, recombinant OB-R molecules can be reconstituted into liposomes for delivery to target cells.

Oligonucleotide sequences including anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of the OB-R variant mRNA are within the scope of the invention. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the OB-R variant nucleotide sequence at nucleotide #2771 and beyond, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of OB-R variant RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their

accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Oligodeoxyribonucleotides can form sequence-specific triple helices by hydrogen bonding to specific complementary sequences in duplexed DNA. Interest in triple helices has focused on the potential biological and therapeutic applications of these structures. Formation of specific triple helices may selectively inhibit the replication and/or gene expression of targeted genes by prohibiting the specific binding of functional trans-acting factors.

Oligonucleotides to be used in triplex helix formation should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Oligonucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich oligonucleotides provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, oligonucleotides may be chosen that are purine-rich, for example, containing a stretch of G residues. These oligonucleotides will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex. Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" oligonucleotide. Switchback oligonucleotides are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

5.4. ACTIVATION OF TYROSINE KINASE PATHWAYS IN OBESITY

Many known class I cytokine receptors initiate cell signaling via Janus kinases (JAKs) (Ihle, 1995, *Nature* 377:591-594; Heldin, 1995, *Cell* 80:213-223; Kishimoto et al, 1994, *Cell* 76:253-62; Ziemiecki et al, 1994, *Trends Cell. Biol.* 4:207-212). JAK1-3 have been shown to bind to conserved sequences termed box1 and box2 (Fukunaga et al., 1991, *EMBO J.* 10:2855-65; Murakami, 1991, *Proc. Natl. Acad. Sci. USA* 88:11349-53). Ligand binding induces a homo- or hetero-dimerization of receptor chains which activates, by phosphorylation, the JAKs. The activated JAKs, in turn, phosphorylate members of the STAT family (Heldin, 1995, *Cell* 80:213-223; Kishimoto et al., *Blood* 86:1243-54; Darnell et al., 1994, *Science* 264:1415-21; Zhong et al, 1994, *Proc.*

Natl. Acad. Sci. USA 91:4806-10; Hou et al., 1994, *Science* 265:1701-6). These phosphorylated STATs ultimately translocate to the nucleus, form transcription complexes, and regulate gene expression. Both box1 and box2 are required for complete signaling in certain systems. (Fukunaga et al., 1991, EMBO J. 10:2855-65; Murakami, 1991, Proc. Natl. Acad. Sci. USA 88:11349-53). The OB-R variant disclosed herein has a typical box1 (from nucleotide #2707-2730) that contains the critical xWxxxPxP amino acid sequence, but it does not contain an obvious box2 nor further downstream sequences that are important for normal receptor activation. Therefore, it is possible to use compounds that activate JAKs to directly activate these pathways for weight regulation without triggering the OB-R.

6. EXAMPLE: MOLECULAR CLONING OF AN OB-R VARIANT

A number of cDNA clones were isolated from a human fetal liver cDNA library (Clontech, Palo Alto, CA), and the DNA sequences of several of these clones were determined. These clones (designated as Hu-B1.219 #4, #33, #34, #1, #8) contained overlapping sequences, which were then compiled into a contiguous nucleotide sequence (Figure 1A-1E). When the deduced amino acid sequence of one such sequence was compared with the sequence of a recently published human OB-R, they were shown to be nearly identical in the extracellular domains with the exception of three amino acids, whereas there existed extensive diversity in their intracellular cytoplasmic domains at the 3' end. The predicted protein sequence contains two FN III domains, each containing a "WS box", which are characteristic of genes of the Class I cytokine receptor family. Therefore, the cDNA disclosed herein encodes an OB-R variant.

When various human tissue RNA were probed with a fragment of this OB-R variant by Northern blot analysis, expression of this molecule was detected in heart, placenta, lung, liver, muscle, pancreas, prostate, ovary, small intestine and brain.

Based on the sequence presented in Figure 1A-1E, the translation initiation site appears at position #97. The sequence encodes an open reading frame up to and including nucleotide #2814. It is believed that the sequence between nucleotides #2629 and #2682 encodes a transmembrane domain. The complete sequence encodes a protein of 906 amino acids.

The sequence of the OB-R variant is identical to the sequence of human OB-R reported by Tartaglia (1995, *Cell* 83:1263-1271) in the transmembrane region and a portion of the intracellular domain up to and including nucleotide #2769, then they diverge at nucleotide #2770 and beyond. In addition, the product of this cDNA is substantially shorter in its intracellular domain than the published human OB-R. These two forms of OB-R may derive from a common precursor mRNA by an alternative splicing mechanism. The sequence in this region is consistent with well known splice junctions.

7. DEPOSIT OF MICROORGANISMS

The following organisms were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

Strain Designation	Accession No.
HuB1.219, #1	75885
HuB1.219, #4	75886
HuB1.219, #33	75888
HuB1.219, #34	75889
HuB1.219, #8	75974

The present invention is not to be limited in scope by the exemplified embodiments, which are intended as illustrations of individual aspects of the invention. Indeed, various modifications for the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications cited herein are incorporated by reference in their entirety.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Snodgrass, H.
Cioffi, Joseph
Zupancic, Thomas
Shafer, Alan

(ii) TITLE OF INVENTION: DETECTION OF A LEPTIN RECEPTOR VARIANT AND METHODS FOR REGULATING OBESITY

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Pennie & Edmonds
(B) STREET: 1155 Avenue of the Americas
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: US
(F) ZIP: 10036-2711

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/588,526
(B) FILING DATE: 18-JAN-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Poissant, Brian M.
(B) REGISTRATION NUMBER: 28,462
(C) REFERENCE/DOCKET NUMBER: 8907-030

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 790-9090
(B) TELEFAX: (212) 869-9741
(C) TELEX: 66141 PENNIE

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2880 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2880

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GCG CGC GCG ACG CAG GTG CCC GAG CCC CGG CCC GCG CCC ATC TCT GCC        48
Ala Arg Ala Thr Gln Val Pro Glu Pro Arg Pro Ala Pro Ile Ser Ala
 1               5                  10                  15

TTC GGT CGA GTT GGA CCC CCG GAT CAA GGT GTA CTT CTC TGA AGT AAG        96
```

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Phe Gly Arg Val Gly Pro Pro Asp Gln Gly Val Leu Leu * Ser Lys
          20                  25                  30

ATG ATT TGT CAA AAA TTC TGT GTG GTT TTG TTA CAT TGG GAA TTT ATT      144
Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe Ile
      35                  40                  45

TAT GTG ATA ACT GCG TTT AAC TTG TCA TAT CCA ATT ACT CCT TGG AGA      192
Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro Trp Arg
  50                  55                  60

TTT AAG TTG TCT TGC ATG CCA CCA AAT TCA ACC TAT GAC TAC TTC CTT      240
Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp Tyr Phe Leu
65                  70                  75                  80

TTG CCT GCT GGA CTC TCA AAG AAT ACT TCA AAT TCG AAT GGA CAT TAT      288
Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser Asn Gly His Tyr
              85                  90                  95

GAG ACA GCT GTT GAA CCT AAG TTT AAT TCA AGT GGT ACT CAC TTT TCT      336
Glu Thr Ala Val Glu Pro Lys Phe Asn Ser Ser Gly Thr His Phe Ser
          100                 105                 110

AAC TTA TCC AAA GCA ACT TTC CAC TGT TGC TTT CGG AGT GAG CAA GAT      384
Asn Leu Ser Lys Ala Thr Phe His Cys Cys Phe Arg Ser Glu Gln Asp
      115                 120                 125

AGA AAC TGC TCC TTA TGT GCA GAC AAC ATT GAA GGA AGG ACA TTT GTT      432
Arg Asn Cys Ser Leu Cys Ala Asp Asn Ile Glu Gly Arg Thr Phe Val
  130                 135                 140

TCA ACA GTA AAT TCT TTA GTT TTT CAA CAA ATA GAT GCA AAC TGG AAC      480
Ser Thr Val Asn Ser Leu Val Phe Gln Gln Ile Asp Ala Asn Trp Asn
145                 150                 155                 160

ATA CAG TGC TGG CTA AAA GGA GAC TTA AAA TTA CTC ATC TGT TAT GTG      528
Ile Gln Cys Trp Leu Lys Gly Asp Leu Lys Leu Phe Ile Cys Tyr Val
              165                 170                 175

GAG TCA TTA TTT AAG AAT CTA TTC AGG AAT TAT AAC TAT AAG GTC CAT      576
Glu Ser Leu Phe Lys Asn Leu Phe Arg Asn Tyr Asn Tyr Lys Val His
          180                 185                 190

CTT TTA TAT GTT CTG CCT GAA GTG TTA GAA GAT TCA CCT CTG GTT CCC      624
Leu Leu Tyr Val Leu Pro Glu Val Leu Glu Asp Ser Pro Leu Val Pro
      195                 200                 205

CAA AAA GGC AGT TTT CAG ATG GTT CAC TGC AAT TGC AGT GTT CAT GAA      672
Gln Lys Gly Ser Phe Gln Met Val His Cys Asn Cys Ser Val His Glu
  210                 215                 220

TGT TGT GAA TGT CTT GTG CCT GTG CCA ACA GCC AAA CTC AAC GAC ACT      720
Cys Cys Glu Cys Leu Val Pro Val Pro Thr Ala Lys Leu Asn Asp Thr
225                 230                 235                 240

CTC CTT ATG TGT TTG AAA ATC ACA TCT GGT GGA GTA ATT TTC CGG TCA      768
Leu Leu Met Cys Leu Lys Ile Thr Ser Gly Gly Val Ile Phe Arg Ser
              245                 250                 255

CCT CTA ATG TCA GTT CAG CCA ATA AAT ATG GTG AAG CCT GAT CCA CCA      816
Pro Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp Pro Pro
          260                 265                 270

TTA GGT TTG CAT ATG GAA ATC ACA GAT GAT GGT AAT TTA AAG ATT TCT      864
Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys Ile Ser
      275                 280                 285
```

```
TGG TCC AGC CCA-CCA TTG GTA CCA TTT CCA CTT CAA TAT CAA GTG AAA          912
Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln Val Lys
    290                 295                 300

TAT TCA GAG AAT TCT ACA ACA GTT ATC AGA GAA GCT GAC AAG ATT GTC          960
Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp Lys Ile Val
305                 310                 315                 320

TCA GCT ACA TCC CTG CTA GTA GAC AGT ATA CTT CCT GGG TCT TCG TAT         1008
Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro Gly Ser Ser Tyr
                325                 330                 335

GAG GTT CAG GTG AGG GGC AAG AGA CTG GAT GGC CCA GGA ATC TGG AGT         1056
Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly Pro Gly Ile Trp Ser
            340                 345                 350

GAC TGG AGT ACT CCT CGT GTC TTT ACC ACA CAA GAT GTC ATA TAC TTT         1104
Asp Trp Ser Thr Pro Arg Val Phe Thr Thr Gln Asp Val Ile Tyr Phe
        355                 360                 365

CCA CCT AAA ATT CTG ACA AGT GTT GGG TCT AAT GTT TCT TTT CAC TGC         1152
Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn Val Ser Phe His Cys
    370                 375                 380

ATC TAT AAG AAG GAA AAC AAG ATT GTT CCC TCA AAA GAG ATT GTT TGG         1200
Ile Tyr Lys Lys Glu Asn Lys Ile Val Pro Ser Lys Glu Ile Val Trp
385                 390                 395                 400

TGG ATG AAT TTA GCT GAG AAA ATT CCT CAA AGC CAG TAT GAT GTT GTG         1248
Trp Met Asn Leu Ala Glu Lys Ile Pro Gln Ser Gln Tyr Asp Val Val
                405                 410                 415

AGT GAT CAT GTT AGC AAA GTT ACT TTT TTC AAT CTG AAT GAA ACC AAA         1296
Ser Asp His Val Ser Lys Val Thr Phe Phe Asn Leu Asn Glu Thr Lys
            420                 425                 430

CCT CGA GGA AAG TTT ACC TAT GAT GCA GTG TAC TGC TGC AAT GAA CAT         1344
Pro Arg Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His
        435                 440                 445

GAA TGC CAT CAT CGC TAT GCT GAA TTA TAT GTG ATT GAT GTC AAT ATC         1392
Glu Cys His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile
    450                 455                 460

AAT ATC TCA TGT GAA ACT GAT GGG TAC TTA ACT AAA ATG ACT TGC AGA         1440
Asn Ile Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg
465                 470                 475                 480

TGG TCA ACC AGT ACA ATC CAG TCA CTT GCG GAA AGC ACT TTG CAA TTG         1488
Trp Ser Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu
                485                 490                 495

AGG TAT CAT AGG AGC AGC CTT TAC TGT TCT GAT ATT CCA TCT ATT CAT         1536
Arg Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His
            500                 505                 510

CCC ATA TCT GAG CCC AAA GAT TGC TAT TTG CAG AGT GAT GGT TTT TAT         1584
Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe Tyr
        515                 520                 525

GAA TGC ATT TTC CAG CCA ATC TTC CTA TTA TCT GGC TAC ACA ATG TGG         1632
Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp
    530                 535                 540

ATT AGG ATC AAT CAC TCT CTA GGT TCA CTT GAC TCT CCA CCA ACA TGT         1680
Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys
545                 550                 555                 560
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GTC CTT CCT GAT TCT GTG GTG AAG CCA CTG CCT CCA TCC AGT GTG AAA      1728
Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Ser Val Lys
                565                 570                 575

CGA GAA ATT ACT ATA AAC ATT GGA TTA TTG AAA ATA TCT TGG GAA AAG      1776
Arg Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys Ile Ser Trp Glu Lys
            580                 585                 590

CCA GTC TTT CCA GAG AAT AAC CTT CAA TTC CAG ATT CGC TAT GGT TTA      1824
Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu
        595                 600                 605

AGT GGA AAA GAA GTA CAA TGG AAG ATG TAT GAG GTT TAT GAT CGA AAA      1872
Ser Gly Lys Glu Val Gln Trp Lys Met Tyr Glu Val Tyr Asp Arg Lys
    610                 615                 620

TCA AAA TCT GTC AGT CTC CCA GTT CCA GAC TTG TGT GCA GTC TAT GCT      1920
Ser Lys Ser Val Ser Leu Pro Val Pro Asp Leu Cys Ala Val Tyr Ala
625                 630                 635                 640

GTT CAG GTG CGC TGT AAG AGG CTA GAT GGA CTG GGA TAT TGG AGT AAT      1968
Val Gln Val Arg Cys Lys Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn
                645                 650                 655

TGG AGC AAT CCA GCC TAC ACA GTT GTC ATG GAT ATA AAA GTT CCT ATG      2016
Trp Ser Asn Pro Ala Tyr Thr Val Val Met Asp Ile Lys Val Pro Met
            660                 665                 670

AGA GGA CCT GAA TTT TGG AGA ATA ATT AAT GGA GAT ACT ATG AAA AAG      2064
Arg Gly Pro Glu Phe Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys
        675                 680                 685

GAG AAA AAT GTC ACT TTA CTT TGG AAG CCC CTG ATG AAA AAT GAC TCA      2112
Glu Lys Asn Val Thr Leu Leu Trp Lys Pro Leu Met Lys Asn Asp Ser
    690                 695                 700

TTG TGC AGT GTT CAG AGA TAT GTG ATA AAC CAT CAT ACT TCC TGC AAT      2160
Leu Cys Ser Val Gln Arg Tyr Val Ile Asn His His Thr Ser Cys Asn
705                 710                 715                 720

GGA ACA TGG TCA GAA GAT GTG GGA AAT CAC ACG AAA TTC ACT TTC CTG      2208
Gly Thr Trp Ser Glu Asp Val Gly Asn His Thr Lys Phe Thr Phe Leu
                725                 730                 735

TGG ACA GAG CAA GCA CAT ACT GTT ACG GTT CTG GCC ATC AAT TCA ATT      2256
Trp Thr Glu Gln Ala His Thr Val Thr Val Leu Ala Ile Asn Ser Ile
            740                 745                 750

GGT GCT TCT GTT GCA AAT TTT AAT TTA ACC TTT TCA TGG CCT ATG AGC      2304
Gly Ala Ser Val Ala Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser
        755                 760                 765

AAA GTA AAT ATC GTG CAG TCA CTC AGT GCT TAT CCT TTA AAC AGC AGT      2352
Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn Ser Ser
    770                 775                 780

TGT GTG ATT GTT TCC TGG ATA CTA TCA CCC AGT GAT TAC AAG CTA ATG      2400
Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr Lys Leu Met
785                 790                 795                 800

TAT TTT ATT ATT GAG TGG AAA AAT CTT AAT GAA GAT GGT GAA ATA AAA      2448
Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp Gly Glu Ile Lys
                805                 810                 815

TGG CTT AGA ATC TCT TCA TCT GTT AAG AAG TAT TAT ATC CAT GAT CAT      2496
Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr Tyr Ile His Asp His
            820                 825                 830
```

```
TTT ATC CCC ATT GAG AAG TAC CAG TTC AGT CTT TAC CCA ATA TTT ATG      2544
Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Ile Phe Met
        835                 840                 845

GAA GGA GTG GGA AAA CCA AAG ATA ATT AAT AGT TTC ACT CAA GAT GAT      2592
Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Ser Phe Thr Gln Asp Asp
    850                 855                 860

ATT GAA AAA CAC CAG AGT GAT GCA GGT TTA TAT GTA ATT GTG CCA GTA      2640
Ile Glu Lys His Gln Ser Asp Ala Gly Leu Tyr Val Ile Val Pro Val
865                 870                 875                 880

ATT ATT TCC TCT TCC ATC TTA TTG CTT GGA ACA TTA TTA ATA TCA CAC      2688
Ile Ile Ser Ser Ser Ile Leu Leu Leu Gly Thr Leu Leu Ile Ser His
                885                 890                 895

CAA AGA ATG AAA AAG CTA TTT TGG GAA GAT GTT CCG AAC CCC AAG AAT      2736
Gln Arg Met Lys Lys Leu Phe Trp Glu Asp Val Pro Asn Pro Lys Asn
            900                 905                 910

TGT TCC TGG GCA CAA GGA CCT AAT TTT CAG AAG AAA ATG CCT GGC ACA      2784
Cys Ser Trp Ala Gln Gly Pro Asn Phe Gln Lys Lys Met Pro Gly Thr
        915                 920                 925

AAG GAA CTA CTG GGT GGA GGT TGG TTG ACT TAG GAA ATG CTT GTG AAG      2832
Lys Glu Leu Leu Gly Gly Gly Trp Leu Thr  *  Glu Met Leu Val Lys
    930                 935                 940

CTA CGT CCT ACC TCG TGC GCA CCT GCT CTC CCT GAG GTG TGC ACA ATG      2880
Leu Arg Pro Thr Ser Cys Ala Pro Ala Leu Pro Glu Val Cys Thr Met
945                 950                 955                 960
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ala Arg Ala Thr Gln Val Pro Glu Pro Arg Pro Ala Pro Ile Ser Ala
  1               5                  10                  15

Phe Gly Arg Val Gly Pro Pro Asp Gln Gly Val Leu Leu
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 908 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ser Lys Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu
  1               5                  10                  15

Phe Ile Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro
```

```
                20                  25                  30

Trp Arg Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp Tyr
        35                  40                  45

Phe Leu Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser Asn Gly
     50                  55                  60

His Tyr Glu Thr Ala Val Glu Pro Lys Phe Asn Ser Ser Gly Thr His
 65                  70                  75                  80

Phe Ser Asn Leu Ser Lys Ala Thr Phe His Cys Cys Phe Arg Ser Glu
                85                  90                  95

Gln Asp Arg Asn Cys Ser Leu Cys Ala Asp Asn Ile Glu Gly Arg Thr
            100                 105                 110

Phe Val Ser Thr Val Asn Ser Leu Val Phe Gln Gln Ile Asp Ala Asn
        115                 120                 125

Trp Asn Ile Gln Cys Trp Leu Lys Gly Asp Leu Lys Leu Phe Ile Cys
    130                 135                 140

Tyr Val Glu Ser Leu Phe Lys Asn Leu Phe Arg Asn Tyr Asn Tyr Lys
145                 150                 155                 160

Val His Leu Leu Tyr Val Leu Pro Glu Val Leu Glu Asp Ser Pro Leu
                165                 170                 175

Val Pro Gln Lys Gly Ser Phe Gln Met Val His Cys Asn Cys Ser Val
            180                 185                 190

His Glu Cys Cys Glu Cys Leu Val Pro Val Pro Thr Ala Lys Leu Asn
        195                 200                 205

Asp Thr Leu Leu Met Cys Leu Lys Ile Thr Ser Gly Gly Val Ile Phe
    210                 215                 220

Arg Ser Pro Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp
225                 230                 235                 240

Pro Pro Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys
                245                 250                 255

Ile Ser Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln
            260                 265                 270

Val Lys Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp Lys
        275                 280                 285

Ile Val Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro Gly Ser
    290                 295                 300

Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly Pro Gly Ile
305                 310                 315                 320

Trp Ser Asp Trp Ser Thr Pro Arg Val Phe Thr Thr Gln Asp Val Ile
                325                 330                 335

Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn Val Ser Phe
            340                 345                 350

His Cys Ile Tyr Lys Lys Glu Asn Lys Ile Val Pro Ser Lys Glu Ile
        355                 360                 365
```

Val Trp Trp Met Asn Leu Ala Glu Lys Ile Pro Gln Ser Gln Tyr Asp
370 375 380

Val Val Ser Asp His Val Ser Lys Val Thr Phe Phe Asn Leu Asn Glu
385 390 395 400

Thr Lys Pro Arg Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn
405 410 415

Glu His Glu Cys His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val
420 425 430

Asn Ile Asn Ile Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr
435 440 445

Cys Arg Trp Ser Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu
450 455 460

Gln Leu Arg Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser
465 470 475 480

Ile His Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly
485 490 495

Phe Tyr Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr
500 505 510

Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro
515 520 525

Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Ser
530 535 540

Val Lys Arg Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys Ile Ser Trp
545 550 555 560

Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr
565 570 575

Gly Leu Ser Gly Lys Glu Val Gln Trp Lys Met Tyr Glu Val Tyr Asp
580 585 590

Arg Lys Ser Lys Ser Val Ser Leu Pro Val Pro Asp Leu Cys Ala Val
595 600 605

Tyr Ala Val Gln Val Arg Cys Lys Arg Leu Asp Gly Leu Gly Tyr Trp
610 615 620

Ser Asn Trp Ser Asn Pro Ala Tyr Thr Val Val Met Asp Ile Lys Val
625 630 635 640

Pro Met Arg Gly Pro Glu Phe Trp Arg Ile Ile Asn Gly Asp Thr Met
645 650 655

Lys Lys Glu Lys Asn Val Thr Leu Leu Trp Lys Pro Leu Met Lys Asn
660 665 670

Asp Ser Leu Cys Ser Val Gln Arg Tyr Val Ile Asn His His Thr Ser
675 680 685

Cys Asn Gly Thr Trp Ser Glu Asp Val Gly Asn His Thr Lys Phe Thr
690 695 700

Phe Leu Trp Thr Glu Gln Ala His Thr Val Thr Val Leu Ala Ile Asn
705 710 715 720

Ser Ile Gly Ala Ser Val Ala Asn Phe Asn Leu Thr Phe Ser Trp Pro

```
                725                 730                 735

Met Ser Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn
            740                 745                 750

Ser Ser Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr Lys
        755                 760                 765

Leu Met Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp Gly Glu
    770                 775                 780

Ile Lys Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr Tyr Ile His
785                 790                 795                 800

Asp His Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Ile
                805                 810                 815

Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Ser Phe Thr Gln
            820                 825                 830

Asp Asp Ile Glu Lys His Gln Ser Asp Ala Gly Leu Tyr Val Ile Val
        835                 840                 845

Pro Val Ile Ile Ser Ser Ser Ile Leu Leu Leu Gly Thr Leu Leu Ile
    850                 855                 860

Ser His Gln Arg Met Lys Lys Leu Phe Trp Glu Asp Val Pro Asn Pro
865                 870                 875                 880

Lys Asn Cys Ser Trp Ala Gln Gly Pro Asn Phe Gln Lys Lys Met Pro
                885                 890                 895

Gly Thr Lys Glu Leu Leu Gly Gly Gly Trp Leu Thr
            900                 905
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Glu Met Leu Val Lys Leu Arg Pro Thr Ser Cys Ala Pro Ala Leu Pro
  1               5                  10                  15

Glu Val Cys Thr Met
            20
```

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 16, lines 17-37 of the description *

A. IDENTIFICATION OF DEPOSIT *

Further deposits are identified on an additional sheet *

Name of depositary institution *

American Type Culture Collection

Address of depositary institution (including postal code and country) *

12301 Parklawn Drive
Rockville, MD 20852
US

Date of deposit * September 14, 1994 Accession Number * 75885

B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau *

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

International Application No: PCT/ /

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive
Rockville, MD 20852
US

Accession No.	Date of Deposit
75886	September 14, 1994
75888	September 14, 1994
75889	September 14, 1994
75974	December 14, 1994

WHAT IS CLAIMED IS:

1. A method for detecting a defective OB-R in cells comprising:

(a) extracting RNA from a cell population;

(b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted in Figure 1A-1E; and

(c) detecting hybridization of the RNA with the oligonucleotide.

2. The method of Claim 1 in which the cell population is obtained from the brain.

3. The method of Claim 1 in which the cell population is obtained from the lung.

4. The method of Claim 1 in which the cell population is obtained from the kidney.

5. The method of Claim 1 in which the oligonucleotide is derived from nucleotide residue #2770 and beyond in the sequence depicted in Figure 1A-1E.

6. A method for treating obesity, comprising administering to an individual an effective amount of an agent capable of inhibiting expression of an OB-R variant gene.

7. The method of Claim 6 in which the OB-R variant gene further comprises the sequence of Figure 1A-1E or which is capable of selectively hybridizing to it.

8. The method of Claim 7 in which the agent is an antisense molecule complementary to mRNA encoded by the sequence of Figure 1A-1E.

9. The method of Claim 7 in which the agent is a ribozyme molecule specific for mRNA encoded by the sequence of Figure 1A-1E.

10. The method of Claim 7 in which the agent is a triple helix component.

11. A method for identifying a compound capable of supplementing biological activity of leptin, comprising:

(a) incubating host cell expressing an OB-R variant with leptin;

(b) incubating a portion of the leptin-treated cells with a test compound; and

(c) comparing activation signal in the cells treated in step (b) with cells treated in step (a);

thereby determining whether the compound augments activation of the OB-R variant by leptin.

12. The method of Claim 11 in which the OB-R variant is encoded by the sequence depicted in Figure 1A-1E.

```
                9                  18                  27                  36                  45                  54
GCG CGC GCG ACG CAG GTG CCC GAG CCC CGG CCC GCG CCC ATC TCT GCC TTC GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   R   A   T   Q   V   P   E   P   R   P   A   P   I   S   A   F   G

               63                  72                  81                  90                  99                 108
CGA GTT GGA CCC CCG GAT CAA GGT GTA CTT CTC TGA AGT AAG ATG ATT TGT CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   V   G   P   P   D   Q   G   V   L   L   *   S   K   M   I   C   Q

              117                 126                 135                 144                 153                 162
AAA TTC TGT GTG GTT TTG TTA CAT TGG GAA TTT ATT TAT GTG ATA ACT GCG TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   F   C   V   V   L   L   H   W   E   F   I   Y   V   I   T   A   F

              171                 180                 189                 198                 207                 216
AAC TTG TCA TAT CCA ATT ACT CCT TGG AGA TTT AAG TTG TCT TGC ATG CCA CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   L   S   Y   P   I   T   P   W   R   F   K   L   S   C   M   P   P

              225                 234                 243                 252                 261                 270
AAT TCA ACC TAT GAC TAC TTC CTT TTG CCT GCT GGA CTC TCA AAG AAT ACT TCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   S   T   Y   D   Y   F   L   L   P   A   G   L   S   K   N   T   S

              279                 288                 297                 306                 315                 324
AAT TCG AAT GGA CAT TAT GAG ACA GCT GTT GAA CCT AAG TTT AAT TCA AGT GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   S   N   G   H   Y   E   T   A   V   E   P   K   F   N   S   S   G

              333                 342                 351                 360                 369                 378
ACT CAC TTT TCT AAC TTA TCC AAA GCA ACT TTC CAC TGT TGC TTT CGG AGT GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   H   F   S   N   L   S   K   A   T   F   H   C   C   F   R   S   E

              387                 396                 405                 414                 423                 432
CAA GAT AGA AAC TGC TCC TTA TGT GCA GAC AAC ATT GAA GGA AGG ACA TTT GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   D   R   N   C   S   L   C   A   D   N   I   E   G   R   T   F   V

              441                 450                 459                 468                 477                 486
TCA ACA GTA AAT TCT TTA GTT TTT CAA CAA ATA GAT GCA AAC TGG AAC ATA CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   T   V   N   S   L   V   F   Q   Q   I   D   A   N   W   N   I   Q

              495                 504                 513                 522                 531                 540
TGC TGG CTA AAA GGA GAC TTA AAA TTA TTC ATC TGT TAT GTG GAG TCA TTA TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   W   L   K   G   D   L   K   L   F   I   C   Y   V   E   S   L   F

              549                 558                 567                 576                 585                 594
AAG AAT CTA TTC AGG AAT TAT AAC TAT AAG GTC CAT CTT TTA TAT GTT CTG CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   N   L   F   R   N   Y   N   Y   K   V   H   L   L   Y   V   L   P

              603                 612                 621                 630                 639                 648
GAA GTG TTA GAA GAT TCA CCT CTG GTT CCC CAA AAA GGC AGT TTT CAG ATG GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   V   L   E   D   S   P   L   V   P   Q   K   G   S   F   Q   M   V
```

Figure 1 A

```
          657                 666                 675                 684                 693                 702
CAC TGC AAT TGC AGT GTT CAT GAA TGT TGT GAA TGT CTT GTG CCT GTG CCA ACA
H   C   N   C   S   V   H   E   C   C   E   C   L   V   P   V   P   T

          711                 720                 729                 738                 747                 756
GCC AAA CTC AAC GAC ACT CTC CTT ATG TGT TTG AAA ATC ACA TCT GGT GGA GTA
A   K   L   N   D   T   L   L   M   C   L   K   I   T   S   G   G   V

          765                 774                 783                 792                 801                 810
ATT TTC CGG TCA CCT CTA ATG TCA GTT CAG CCC ATA AAT ATG GTG AAG CCT GAT
I   F   R   S   P   L   M   S   V   Q   P   I   N   M   V   K   P   D

          819                 828                 837                 846                 855                 864
CCA CCA TTA GGT TTG CAT ATG GAA ATC ACA GAT GAT GGT AAT TTA AAG ATT TCT
P   P   L   G   L   H   M   E   I   T   D   D   G   N   L   K   I   S

          873                 882                 891                 900                 909                 918
TGG TCC AGC CCA CCA TTG GTA CCA TTT CCA CTT CAA TAT CAA GTG AAA TAT TCA
W   S   S   P   P   L   V   P   F   P   L   Q   Y   Q   V   K   Y   S

          927                 936                 945                 954                 963                 972
GAG AAT TCT ACA ACA GTT ATC AGA GAA GCT GAC AAG ATT GTC TCA GCT ACA TCC
E   N   S   T   T   V   I   R   E   A   D   K   I   V   S   A   T   S

          981                 990                 999                1008                1017                1026
CTG CTA GTA GAC AGT ATA CTT CCT GGG TCT TCG TAT GAG GTT CAG GTG AGG GGC
L   L   V   D   S   I   L   P   G   S   S   Y   E   V   Q   V   R   G

         1035                1044                1053                1062                1071                1080
AAG AGA CTG GAT GGC CCA GGA ATC TGG AGT GAC TGG AGT ACT CCT CGT GTC TTT
K   R   L   D   G   P   G   I   W   S   D   W   S   T   P   R   V   F

         1089                1098                1107                1116                1125                1134
ACC ACA CAA GAT GTC ATA TAC TTT CCA CCT AAA ATT CTG ACA AGT GTT GGG TCT
T   T   Q   D   V   I   Y   F   P   P   K   I   L   T   S   V   G   S

         1143                1152                1161                1170                1179                1188
AAT GTT TCT TTT CAC TGC ATC TAT AAG AAG GAA AAC AAG ATT GTT CCC TCA AAA
N   V   S   F   H   C   I   Y   K   K   E   N   K   I   V   P   S   K

         1197                1206                1215                1224                1233                1242
GAG ATT GTT TGG TGG ATG AAT TTA GCT GAG AAA ATT CCT CAA AGC CAG TAT GAT
E   I   V   W   W   M   N   L   A   E   K   I   P   Q   S   Q   Y   D

         1251                1260                1269                1278                1287                1296
GTT GTG AGT GAT CAT GTT AGC AAA GTT ACT TTT TTC AAT CTG AAT GAA ACC AAA
V   V   S   D   H   V   S   K   V   T   F   F   N   L   N   E   T   K

         1305                1314                1323                1332                1341                1350
CCT CGA GGA AAG TTT ACC TAT GAT GCA GTG TAC TGC TGC AAT GAA CAT GAA TGC
P   R   G   K   F   T   Y   D   A   V   Y   C   C   N   E   H   E   C
```

Figure 1B

```
          1359                1368                1377                1386                1395                1404
CAT CAT CGC TAT GCT GAA TTA TAT GTG ATT GAT GTC AAT ATC AAT ATC TCA TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   H   R   Y   A   E   L   Y   V   I   D   V   N   I   N   I   S   C

          1413                1422                1431                1440                1449                1458
GAA ACT GAT GGG TAC TTA ACT AAA ATG ACT TGC AGA TGG TCA ACC AGT ACA ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   T   D   G   Y   L   T   K   M   T   C   R   W   S   T   S   T   I

          1467                1476                1485                1494                1503                1512
CAG TCA CTT GCG GAA AGC ACT TTG CAA TTG AGG TAT CAT AGG AGC AGC CTT TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   S   L   A   E   S   T   L   Q   L   R   Y   H   R   S   S   L   Y

          1521                1530                1539                1548                1557                1566
TGT TCT GAT ATT CCA TCT ATT CAT CCC ATA TCT GAG CCC AAA GAT TGC TAT TTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   S   D   I   P   S   I   H   P   I   S   E   P   K   D   C   Y   L

          1575                1584                1593                1602                1611                1620
CAG AGT GAT GGT TTT TAT GAA TGC ATT TTC CAG CCA ATC TTC CTA TTA TCT GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   S   D   G   F   Y   E   C   I   F   Q   P   I   F   L   L   S   G

          1629                1638                1647                1656                1665                1674
TAC ACA ATG TGG ATT AGG ATC AAT CAC TCT CTA GGT TCA CTT GAC TCT CCA CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   T   M   W   I   R   I   N   H   S   L   G   S   L   D   S   P   P

          1683                1692                1701                1710                1719                1728
ACA TGT GTC CTT CCT GAT TCT GTG GTG AAG CCA CTG CCT CCA TCC AGT GTG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   C   V   L   P   D   S   V   V   K   P   L   P   P   S   S   V   K

          1737                1746                1755                1764                1773                1782
GCA GAA ATT ACT ATA AAC ATT GGA TTA TTG AAA ATA TCT TGG GAA AAG CCA GTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   E   I   T   I   N   I   G   L   L   K   I   S   W   E   K   P   V

          1791                1800                1809                1818                1827                1836
TTT CCA GAG AAT AAC CTT CAA TTC CAG ATT CGC TAT GGT TTA AGT GGA AAA GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   P   E   N   N   L   Q   F   Q   I   R   Y   G   L   S   G   K   E

          1845                1854                1863                1872                1881                1890
GTA CAA TGG AAG ATG TAT GAG GTT TAT GAT GCA AAA TCA AAA TCT GTC AGT CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   Q   W   K   M   Y   E   V   Y   D   A   K   S   K   S   V   S   L

          1899                1908                1917                1926                1935                1944
CCA GTT CCA GAC TTG TGT GCA GTC TAT GCT GTT CAG GTG CGC TGT AAG AGG CTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   V   P   D   L   C   A   V   Y   A   V   Q   V   R   C   K   R   L

          1953                1962                1971                1980                1989                1998
GAT GGA CTG GGA TAT TGG AGT AAT TGG AGC AAT CCA GCC TAC ACA GTT GTC ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   G   L   G   Y   W   S   N   W   S   N   P   A   Y   T   V   V   M

          2007                2016                2025                2034                2043                2052
GAT ATA AAA GTT CCT ATG AGA GGA CCT GAA TTT TGG AGA ATA ATT AAT GGA GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   I   K   V   P   M   R   G   P   E   F   W   R   I   I   N   G   D
```

Figure 1C

```
      2061       2070       2079       2088       2097       2106
ACT ATG AAA AAG GAG AAA AAT GTC ACT TTA CTT TGG AAG CCC CTG ATG AAA AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   M   K   K   E   K   N   V   T   L   L   W   K   P   L   M   K   N

      2115       2124       2133       2142       2151       2160
GAC TCA TTG TGC AGT GTT CAG AGA TAT GTG ATA AAC CAT CAT ACT TCC TGC AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   S   L   C   S   V   Q   R   Y   V   I   N   H   H   T   S   C   N

      2169       2178       2187       2196       2205       2214
GGA ACA TGG TCA GAA GAT GTG GGA AAT CAC ACG AAA TTC ACT TTC CTG TGG ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   T   W   S   E   D   V   G   N   H   T   K   F   T   F   L   W   T

      2223       2232       2241       2250       2259       2268
GAG CAA GCA CAT ACT GTT ACG GTT CTG GCC ATC AAT TCA ATT GGT GCT TCT GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   Q   A   H   T   V   T   V   L   A   I   N   S   I   G   A   S   V

      2277       2286       2295       2304       2313       2322
GCA AAT TTT AAT TTA ACC TTT TCA TGG CCT ATG AGC AAA GTA AAT ATC GTG CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   N   F   N   L   T   F   S   W   P   M   S   K   V   N   I   V   Q

      2331       2340       2349       2358       2367       2376
TCA CTC AGT GCT TAT CCT TTA AAC AGC AGT TGT GTG ATT GTT TCC TGG ATA CTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   L   S   A   Y   P   L   N   S   S   C   V   I   V   S   W   I   L

      2385       2394       2403       2412       2421       2430
TCA CCC AGT GAT TAC AAG CTA ATG TAT TTT ATT ATT GAG TGG AAA AAT CTT AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   P   S   D   Y   K   L   M   Y   F   I   I   E   W   K   N   L   N

      2439       2448       2457       2466       2475       2484
GAA GAT GGT GAA ATA AAA TGG CTT AGA ATC TCT TCA TCT GTT AAG AAG TAT TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   D   G   E   I   K   W   L   R   I   S   S   S   V   K   K   Y   Y

      2493       2502       2511       2520       2529       2538
ATC CAT GAT CAT TTT ATC CCC ATT GAG AAG TAC CAG TTC AGT CTT TAC CCA ATA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   H   D   H   F   I   P   I   E   K   Y   Q   F   S   L   Y   P   I

      2547       2556       2565       2574       2583       2592
TTT ATG GAA GGA GTG GGA AAA CCA AAG ATA ATT AAT AGT TTC ACT CAA GAT GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   M   E   G   V   G   K   P   K   I   I   N   S   F   T   Q   D   D

      2601       2610       2619       2628       2637       2646
ATT GAA AAA CAC CAG AGT GAT GCA GGT TTA TAT GTA ATT GTG CCA GTA ATT ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   E   K   H   Q   S   D   A   G   L   Y   V   I   V   P   V   I   I

      2655       2664       2673       2682       2691       2700
TCC TCT TCC ATC TTA TTG CTT GGA ACA TTA TTA ATA TCA CAC CAA AGA ATG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   S   S   I   L   L   L   G   T   L   L   I   S   H   Q   R   M   K

      2709       2718       2727       2736       2745       2754
AAG CTA TTT TGG GAA GAT GTT CCG AAC CCC AAG AAT TGT TCC TGG GCA CAA GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   L   F   W   E   D   V   P   N   P   K   N   C   S   W   A   Q   G
```

Figure 1D

```
          2763                2772                2781                2790                2799                2808
CTT AAT TTT CAG AAG AAA ATG CCT GGC ACA AAG GAA CTA CTG GGT GGA GGT TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   N   F   Q   K   K   M   P   G   T   K   E   L   L   G   G   G   W

          2817                2826                2835                2844                2853                2862
TTG ACT TAG GAA ATG CTT GTG AAG CTA CGT CCT ACC TCG TGC GCA CCT GCT CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   T   *   E   M   L   V   K   L   R   P   T   S   C   A   P   A   L

          2871                2880
CCT GAG GTG TGC ACA ATG 3'
--- --- --- --- --- ---
 P   E   V   C   T   M
```

Figure 1E